was admitted to another ward at that time, and discharged some weeks later. She was only admitted to the ward from which the wounds infected with the resistant strain were first detected after the nephrectomy patient was discharged and the nurse's lesion had healed.

The results of the tube sensitivity-tests on the six strains are given in the Table. Coagulase-positive staphylococci are normally sensitive to 1.25–2.5 μg./ml. celbenin.[1]

It is interesting that the four strains from the eczematous patient show different minimal inhibitory concentrations but that the two colonies tested from each culture showed the same sensitivity. This has not been explored further. Assay of residual celbenin in broth after growth of strain 13137 failed to demonstrate destruction of the antibiotic.

No direct connexion between the strain isolated from the eczematous patient in July and the two strains isolated in October could be demonstrated. Nurse B was not on duty in the out-patient department or the ward to which the patient was admitted in July, and she was not a nasal carrier at the end of October when her lesion had healed. It seems, however, very likely that the strain isolated from patient C in July was in fact the same as that isolated from patient A and nurse B in October, especially as the original patient with the infected eczema is a regular hospital attender, and this strain has been isolated from her nose three times over a three-month period and from her skin on the only occasion this was swabbed.

Celbenin had been used for one patient in this hospital. This was during October, but the patient, a diabetic with multiple boils, was treated in a side ward by a different nursing staff, and the infecting organism was a staphylococcus of phage-type 80/81. It does not seem, therefore, that the resistant strains could have arisen as a result of this therapy.

It is interesting that the celbenin-resistant strains belong to Group III phage-pattern, as it is in this group that the first staphylococci resistant to each new antibiotic have arisen. Even supposing the three strains isolated represent two naturally occurring strains, the frequency of resistant strains is only 0.036% in this series. Several other workers have failed to find resistant strains in the large number of strains tested.[2 3] Of the 4,340 strains from routine material in this series the great majority were penicillin-resistant hospital strains, and many of them were resistant to several antibiotics. The finding of these strains does not therefore detract from the very great value of celbenin, but the fact that the occasional resistant strain does exist should be borne in mind.

It is well known that patients with infected skin can be dangerous sources of infection in hospitals, and the finding of just such a patient infected with a celbenin-resistant strain in this instance adds an additional warning.—I am, etc.,

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SIR,—In October, 1960, we received from Dr. M. P. Jevons two single-colony isolates each of two strains of staphylococci which she had found to be resistant to "celbenin." These are the cultures referred to in her letter above and designated 13136a and c and 13137a and c. Subsequently, we also received from Dr. Jevons the original cultures 13136 and 13137 which had been sent to Colindale for phage-typing. The origin of these cultures is described in her letter.

We tested the sensitivity of these cultures to celbenin by serial dilution in agar and in nutrient broth. The broth dilutions were inoculated with one drop of an overnight broth-culture and the agar dilutions by streaking with a loopful from an overnight broth culture. The tests were incubated at 37° C. for 24 hours. Both types of tests gave the same results. With culture 13136, and also the isolates from 13136, the minimum inhibitory concentration (M.I.C.) of celbenin was 5.0 μg./ml. The normal M.I.C. for penicillin-resistant staphylococci in these tests is 2.0 μg./ml. With culture 13137, and one of the isolates from 13137, the M.I.C. was 25 μg./ml. In every case the end-point was sharp. In the broth-cultures growth was heavy up to the M.I.C. value, but with no visible growth beyond this concentration. On further incubation, however, a very different result was obtained. After 48 hours' incubation all the cultures showed heavy growth in broth culture up to and including a concentration of 250 μg./ml. Strains were isolated from this growth in 250 μg./ml. which, on subsequent testing, showed M.I.C. values of 250–500 μg./ml. after overnight incubation.

The cultures 13136 and 13137 thus appeared to be predominantly of a type not appreciably different in sensitivity to celbenin from other penicillin-resistant staphylococci, but in addition there appeared to be a very small proportion which were highly resistant. Plating out large numbers of cells of 13136 and 13137 on agar-containing doubling dilutions of celbenin showed that after incubation overnight at 37° C. only one in approximately 10^7 of the population grew at 12.5 μg./ml. and only one in approximately 10^8 grew at a concentration of 250 μg./ml.

The resistant type isolated from broth cultures containing 250 μg./ml. of celbenin grew well on 7.5% salt agar, gave acid production with mannitol and liquefaction of gelatin, and was coagulase-positive. The culture was also penicillinase-producing, penicillin-resistant, and resistant also to streptomycin and tetracycline, as was the rest of the culture. Phage-typing, which was kindly carried out by Dr. Jevons, showed this resistant strain to be of the same phage-type as the rest of the culture. A pure culture of the resistant strain grew up readily overnight in broth in concentrations of celbenin up to 250 μg./ml. Assay of the celbenin-content of these broths from 1.0–250 μg./ml. after 24 hours' incubation showed no detectable destruction of celbenin compared with uninoculated broths incubated in the same way. The resistance of this culture is therefore not due to celbenin-destruction by a "celbeninase."

Staphylococci resistant to celbenin can, of course, be obtained quite readily *in vitro* by repeated subculture in the presence of the compound. Whether this is of any significance from the clinical point of view, however, is another matter. Resistance of this type also develops very readily to penicillin G, but cultures of this type are never encountered clinically, and, so far, strains resistant to celbenin have not been isolated from patients following treatment with this antibiotic. When celbenin was marketed in September, 1960, no strains of penicillin-resistant *Staphylococcus aureus* had been found which were resistant to celbenin. Since that time very large numbers of strains have been screened in different laboratories in this country and elsewhere. However, the only strains so far reported to be resistant to celbenin are those described in this letter and the accompanying letters. From the total number of strains examined by Dr. Jevons and those covered in the published literature[1–5] the incidence of celbenin-resistant strains of *Staphylococcus aureus* appears to be less than one in 5,000 of the strains encountered in hospitals

to-day. Resistant strains of the type described in this letter are remarkable more for their infrequency than for their existence.—I am, etc.,

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4 Stewart, G. T., Harrison, P. M., and Holt, R. J., *Brit. med. J.*, 1960, **2**, 694.
5 Thompson, R. E. M., Harding, J. W., and Simon, R. D., ibid., 1960, **2**, 708.

SIR,—The new penicillin, BRL 1241 (" celbenin "), has already proved its worth in the treatment of penicillin-resistant staphylococcal infections. But it is now clear that, as judged by laboratory sensitivity tests, there are at least two types of resistance to celbenin in staphylococci. In several laboratories resistant strains have been artificially produced by different methods of subculture in celbenin-containing media.[1-3] We ourselves have now produced variants resistant to at least 500 μg./ml. of the drug from a strain of *Staph. pyogenes* (E3) initially sensitive to 2–5 μg./ml.[2] The second type of resistance, which may be more serious, is reported in the accompanying letter (p. 124) by Dr. Patricia Jevons from the Staphylococcus Reference Laboratory, Colindale. She has investigated the celbenin-sensitivity of several thousand strains of *Staphylococcus pyogenes* isolated mainly from hospital sources from different parts of the country. One small group of strains which were all of the same phage-type and were isolated from three persons in one hospital showed increased resistance ranging from 6 to 25 μg./ml. as compared with the normal range of 2 to 5 μg./ml.

The terms sensitive and resistant can be misleading, especially when only simple sensitivity tests are done, using serial drug-dilutions with a large inoculum of organisms. Often, more information is required about the distribution of resistance within bacterial populations. This can be obtained only by more elaborate experiments in which different inocula are tested against different concentrations of the drug.

We have investigated a number of strains of *Staph. pyogenes* in this way. Among the strains used have been the penicillinase-producing strain E3 from a patient already mentioned and a laboratory-trained variant of this made resistant by repeated subculture in celbenin to 500 μg./ml. of the drug (E3/500), some naturally occurring celbenin-resistant strains kindly provided by Dr. Patricia Jevons (13136, 13137, 14083, 10395) and some variants of 13137 made resistant in this laboratory by subculture in celbenin to 50, 400, and 1,000 μg./ml. of the drug (13137/50, 13137/400, and 13137/1000). With each strain serial tenfold dilutions of broth cultures were inoculated on to agar plates containing different concentrations of celbenin and on to plain nutrient agar. Thus the number of organisms inoculated was known and a rough estimate could be made of the proportion surviving in different concentrations of the drug.

The results may be briefly summarized:

1. With the E3 strain all the inoculated cells survived in 3 μg./ml. of celbenin but none in 6 μg./ml. even when 10^8 cells were inoculated. Strain 13137 from Colindale was quite different. All the cells inoculated survived in 3 μg./ml., about 1 in 10^4 cells survived in 12 μg./ml., while only 1 in 10^7 survived in 100 μg./ml. Two of the other Colindale strains, 13136 and 10395, behaved similarly, but strain 14083, although from the same patient as 10395, was more like a normally sensitive strain.

2. The laboratory-trained resistant variants, E3/500 and 13137/1000, were interesting. When first isolated both strains grew much more slowly even on drug-free medium than their parent strains, but the 13137/1000 variant from the naturally resistant strain 13137 quickly recovered the ability to grow normally on nutrient agar, whereas the E3/500 variant from the naturally sensitive strain E3 still gives poor growth. With both strains, however, all the cells inoculated grow up to high concentrations of celbenin on longer incubation.

3. There has been no evidence so far that any of these strains can destroy celbenin. This has been investigated both by microbiological and chemical assay methods.

These facts are clearly important, but must be viewed in proper perspective. On the one hand, it would be foolish to ignore the facts (1) that strains naturally resistant to celbenin actually existed before the drug was used, and (2) that cultures of *Staph. pyogenes* can be " trained " artificially in the laboratory to high levels of resistance. On the other hand, it should be emphasized (1) that mutants resistant to celbenin were bound to be detected if they were looked for thoroughly enough, and Professor R. E. O. Williams, who with Dr. Jevons initiated this investigation at Colindale, should be congratulated on seizing the opportunity for this presented by the existence of the staphylococcus reference laboratory, (2) that it is the rarity of this resistance rather than its frequency which is remarkable, and (3) that so far there have been no reports of failure due to the development of celbenin-resistant staphylococci in patients treated with it.

We must, of course, be always on the look-out for resistant strains. We must avoid indiscriminate use of the drug which is only likely to increase the chances of such strains spreading. But these warnings should not make us afraid to use the drug where it is properly indicated and to take full advantage of its remarkably effective action against penicillin-resistant staphylococci. —I am, etc.,

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Aetiology of Coronary Disease

SIR,—In a recent paper (November 5, 1960, p. 1329) concerned with the pathogenesis of coronary artery disease, Sir Howard Florey mentioned that some investigators consider the protein content of the diet to be of great importance (possibly as important as the lipid content) in controlling serum lipid levels. I think it is also of interest that over 30 years ago Mills and Necheles[1-4] observed increased coagulability of the blood *in vitro* as well as acceleration of platelet aggregation and disintegration following the ingestion of protein; on the other hand, no such changes were seen after either fat or carbohydrate had been given. Furthermore, it is generally recognized that communities showing the lowest incidence of ischaemic heart disease subsist on diets which contain relatively small amounts of protein (especially animal protein) as well as of fat.[5] Some of us have the impression, too, that